FORAGE & GRAZING LANDS

Growth and Complexity of White Clover Stolons in Response to Biotic and Abiotic Stress

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ABSTRACT

White clover (*Trifolium repens* L.) persists in pastures mainly by stolon growth. Morphologically complex (i.e., highly branched stolons) plants of white clover generally persist longer. We hypothesized that biotic and abiotic stresses limit white clover production on grazing lands by fragmenting plants into smaller, less competitive individuals. We measured changes in the size and structure of plants in a white clover–orchardgrass (*Dactylis glomerata* L.) sward during a drought (1999) and a favorable growing season (2000) in grazed pastures on a southeastern Pennsylvania farm. A natural infestation of clover root curculio [*Sitona hispidulus* (Fabricus)] and blue clover weevil [*Ischnopterapion virens* (Herbst)] provided an opportunity to examine the interaction of abiotic and biotic stress on stolon structure. White clover plants were dug from two orchardgrass ('Pennlate')–white clover ('Will') pastures during April to November 1999 and 2000. Stolon structure and damage from blue weevil and curculio larvae were determined monthly. Drought in 1999 reduced stolon production, branching, and rooting in white clover. Stolon length (cm m^{-2}) in 1999 was 50% of that in 2000. Clover root curculio damaged up to 25% of clover roots and 20 to 40% of stolons were damaged by weevils. Insect damage was greatest on primary stolons. With favorable rainfall during late 1999 and in 2000, white clover recovered from fragmentation and produced nearly twice the stolon length, mass, and density in the next grazing season despite insect damage levels of 10 to 30%. Climate and biotic stresses are the major factors controlling oscillations of white clover stolon density in pastures of the northeastern USA.

WHITE CLOVER is a critical component of pastures in temperate humid grazing lands and persists via clonal growth of stolons and seedling recruitment (Pederson, 1995). Through N fixation, it supplies much of the N needed for growth of itself and other species within the sward. Persistence of white clover depends on many factors such as soil type, slope aspect, water, frequency and extent of grazing and cutting, soil fertility, plant genetics, and insect and pathogen infestation.

Clonally propagated plants such as white clover exploit new niches by fragmentation of existing plants (Brock et al., 1988). Research in New Zealand, the UK, and Europe has shown that fragmentation of white clover growing with perennial ryegrass (*Lolium perenne* L.) occurs mostly in spring, and the new, small, less complex plants (plants with fewer branches) are particularly sensitive to stress at this time (Brock et al., 1988;

Fothergill et al., 1997; Wachendorf et al., 2001a). Research in the northeastern USA, however, indicated that white clover may not follow this pattern in a colder climate. White clover plants in grazed mixed-species pastures in central New York were not smaller and did not have a simpler branching order in spring (Karsten and Fick, 1999). Rather, white clover plants were smaller, less complex, and had fewer roots during and for a short time after a drought in mid-summer. Karsten and Fick (1999) speculated that grazing of white clover during the milder winter climate in New Zealand imposed treading and defoliation stress on white clover, whereas in New York white clover remains dormant and is not grazed during the cold winter. A 30-yr study of environmental effects on the persistence of white clover in Australia concluded that late summer moisture stress was the critical factor limiting white clover persistence (Hutchinson et al., 1995). Soil type and environment had major effects on white clover structure in three environments in Pennsylvania (Sanderson and Elwinger, 2002). Companion grass cultivars also affected the structure of seedling white clover (Sanderson and Elwinger, 1999) but not established white clover plants (Sanderson and Elwinger, 2002).

Biotic stress from insect or disease organisms was not reported to contribute to the seasonal variation in clover structure in the New Zealand, UK, and Wales studies (Brock et al., 1988; Fothergill et al., 1997; Wachendorf et al., 2001a). In the New York research (Karsten and Fick, 1999), however, the authors speculated that fungal pathogens caused white clover plants to fragment. Biotic stress, such as fungal and virus diseases along with insects, can reduce significantly the yield and persistence of white clover (Kilpatrick and Dunn, 1958). Abiotic stresses, along with biotic stresses of pathogens, insects, and grazing livestock, may fragment plants into smaller, less competitive individuals and contribute to species disappearance from grazed swards.

A Palearctic weevil, *Ischnopterapion virens* (Herbst) (the blue weevil), a pest of red clover (*Trifolium pratense* L.) in Europe, recently was discovered as a potential pest of white clover in the northeastern USA. The weevil was first detected in 1994 in pastures of two eastern Pennsylvania dairy farms (Hoebeke et al., 2000). Surveys during 1997 to 1999 showed the presence of the blue weevil in several northeastern states (Byers and Sanderson, 2000). Adults emerge from the host plant in June and feed on clover leaves. The adult weevils aestivate away from fields during the summer months and return to clover in the fall to feed on clover leaves and mate. The adults overwinter in the field near the

base of white and red clover plants. Adults lay eggs in March and the larvae feed inside clover stolons and stems during April and May. Pupation occurs inside stems during the last week of May. Adults chew an exit hole in the stolon and emerge in June (Byers and Sanderson, 2000). The amount of stolon damage caused by the blue weevil and its effect on persistence of white clover stolons is not known.

Another major insect pest of white clover in the northeastern USA is the clover root curculio. Adult curculio weevils feed on white clover leaflets, whereas the larvae feed on roots and root nodules (Murray et al., 1996). Adults overwinter in field borders and lay eggs in the soil in the spring. Larvae attack the root nodules and roots, eventually scarring the taproot. Larval feeding injury enables fungi to penetrate the roots, leading to root rot (Kilpatrick and Dunn, 1958; Quinn and Hower, 1986; Leath and Hower, 1993).

Our earlier research showed that environment (soil type, elevation, and climate) had a much larger effect on white clover stolon structure than cultivar differences within companion grass species or plant density (Sanderson and Elwinger, 2002). That experiment was conducted in small field plots and plants were clipped instead of grazed. Our initial objective in the current experiment was to examine the pattern of white clover stolon development in a grazed sward on a stressful soil type and environment. We hypothesized that white clover plants would be smaller and less complex during summer when heat and drought stress occur, similar to the pattern observed in New York (Karsten and Fick, 1999). We later modified our objective after we noted significant damage to stolons and roots of white clover by the larvae of the clover root curculio and blue weevil. Thus, we documented the effects of biotic and abiotic stresses on white clover stolon growth and complexity.

MATERIALS AND METHODS

The study was conducted on a farm in Berks County, PA. Soil at the farm is a Weikert-Berks shaly silt loam [loamy-skeletal, mixed, active, mesic Lithic (Typic) Dystrudepts]. This soil is well drained, contains a high amount (10 to 50%) of coarse rock fragments (25 to 75 mm in diameter; Barnes and Beard, 1997), and has a low water-holding capacity. Soil pH was 6.4 and available P and K levels to a 15-cm depth averaged 88 and 142 kg ha^{-1}, respectively, in 1999 and 2000. Lime was applied at 2200 kg ha^{-1} in spring 2000. An automated weather station (Campbell Scientific, Logan, Utah) at the site recorded rainfall, air temperature, and soil moisture (15-cm depth in 1999; 15- and 60-cm depths in 2000; Theta Probe ML2x, Delta-T Devices, Cambridge, UK).

Two 0.4-ha paddocks of Pennlate orchardgrass and Will white clover were no-till planted with a Tye Pasture Pleaser drill (Tye Co., Lockney, TX) at 2 and 2.7 kg seed ha^{-1}, respectively, on 19 Sept. 1997. The previous crop was winter wheat (*Triticum aestivum* L.). Glyphosate [N-(phosphonomethyl) glycine] was applied to the wheat stubble at 1.0 kg a.i. ha^{-1} 2 wk before planting. Paddocks were cut for hay, but not grazed, in 1998. Beginning in 1999 and continuing into 2000, paddocks were stocked with 45 to 60 Holstein dairy heifers (*Bos taurus*) for a 1- to 2-d period of stay on a 30 to 45-d rotation interval. Grazing started in late April and ended the

first week of October each year. Paddocks were cut for hay once in June of 1999 and 2000.

Starting in April 1999, we cut five 0.1-m^2 sod pieces from each of the two orchardgrass–white clover paddocks at randomly selected locations shortly before the paddocks were grazed. The sod pieces were cut monthly from April to November in 1999 and 2000. Before cutting the sod pieces, we measured the canopy height of five grass tillers and five white clover petioles within the 0.1 m^2. The sod pieces were cut with a steel-edged quadrat and a spade to a 10-cm depth. Loose soil was gently shaken from the sod and the sod piece was transported to the laboratory. Soil was washed from the sod with water and individual grass and clover plants separated. Roots were trimmed and discarded from the grass plants. The total number of orchardgrass tillers in the sample was counted. Ten tillers were selected at random and the number of live leaves recorded. All tillers were dried at 55°C for 48 h and dry weight recorded.

We separated the white clover stolons into primary stolons and higher order branches. First-order branches originated from the parent stolon and second-order branches originated from first-order branches. We found very few third-order branches at any sampling date and thus ignored them for data analysis purposes. The number of leaves, nodes, and nodes with roots were counted on each stolon class. Leaves were removed and dried separately.

An experienced entomologist (R.A. Byers) rated stolons and roots in the laboratory for root damage by *Sitona* larvae and stolon damage by the blue weevil larvae each month from July to November 1999 and April to November 2000. The analyst split the stolons with a scalpel and measured the length of the feeding tunnels caused by blue weevil larvae. The same analyst visually estimated the percentage of taproot and secondary root surface scarred by *Sitona* larvae. After rating, roots were trimmed and discarded from the stolons. Stolons and leaves were dried at 55°C for 48 h and dry weight recorded.

The two pastures were considered replicates. The MIXED procedure in SAS Institute (1998) was used for statistical analysis. The ANOVA model contained four fixed effects terms: years, months nested within years, pastures, and the years $\times$ pastures interaction. The experimental error term used to test each of these terms was the pastures $\times$ months within years interaction. Data were checked for normality and heteroscedasticity and transformed as necessary. Means and SEs of the transformed data are presented in Tables A1 to A5. Because of the differences in transformations and scales among different variates, and to simplify presentation, we present untransformed data in line graphs in the Results and Discussion. Standard errors are not presented in the line graphs of untransformed data because SEs based on transformed data are not convertible into the corresponding untransformed value.

RESULTS

Weather

Less than 50% of the long-term average summer rainfall occurred in 1999 (Table 1), resulting in a severe drought. Paddocks had very little green plant material during late July and August of 1999 and were not grazed during this time. Soil moisture levels were very low (Fig. 1) and drought stress was evident on this rocky soil. Heavy rains occurred during September 1999 (307 mm vs. 98 mm for the long-term average) as part of the breakup of Hurricane Floyd along the eastern

Table 1. Temperature and rainfall at the farm in Berks County, PA, compared with the long-term average.†

Month	Temperature			Rainfall		
	1999‡	2000	Long-term average	1999‡	2000	Long-term average
		°C			mm	
January		−2.3	−3.3			
February		0	−0.7			
March		7.5	3.9		167	69
April		9.8	10.0		70	98
May	16.5	16.1	15.8	11	120	147
June	21.0	20.4	20.4	73	181	101
July	25.4	21.0	23.3	13	34	112
August	22.3	21.1	22.0	73	71	89
September	18.6	16.6	17.5	307	135	98
October	10.5	12.4	10.9	66	49	90
November	8.2	5.4	5.8	70	60	109
December	2.0	−3.2	−0.6	55	78	76

† Long-term average data are from Waltman et al. (1997) and are for the period 1979 to 1990 at Hamburg, PA, ≈10 km from the study site.
‡ The automated weather station was not installed until May 1999; therefore, temperature and rainfall data are not available before that time.

U.S. coast. This rainfall enabled recovery of the grass and legume paddocks during the fall. Much higher rainfall and slightly lower temperatures in 2000 than in 1999 (Table 1) resulted in greater soil moisture during much of 2000 compared with 1999 (Fig. 1). These two contrasting years provided the opportunity to observe the effects of drought along with insect stress on white clover stolon dynamics.

White Clover Stolon Structure

In 1999, the number, length, and mass of white clover stolons peaked in May (Fig. 2). Primary stolons and first-order branches dominated the white clover popula-
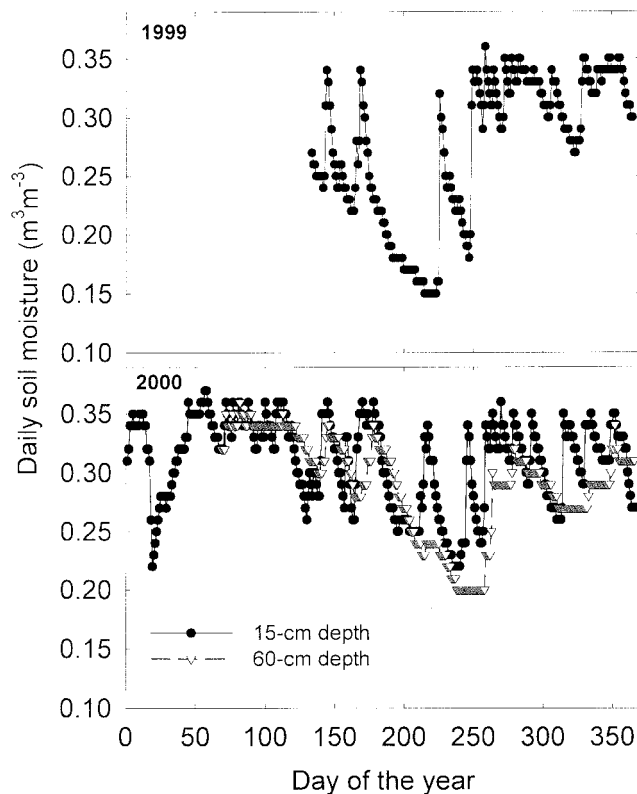

Fig. 1. Soil moisture to the 15-cm depth (1999 and 2000) and 60-cm depth (2000) recorded at the experimental site in Berks County, PA.
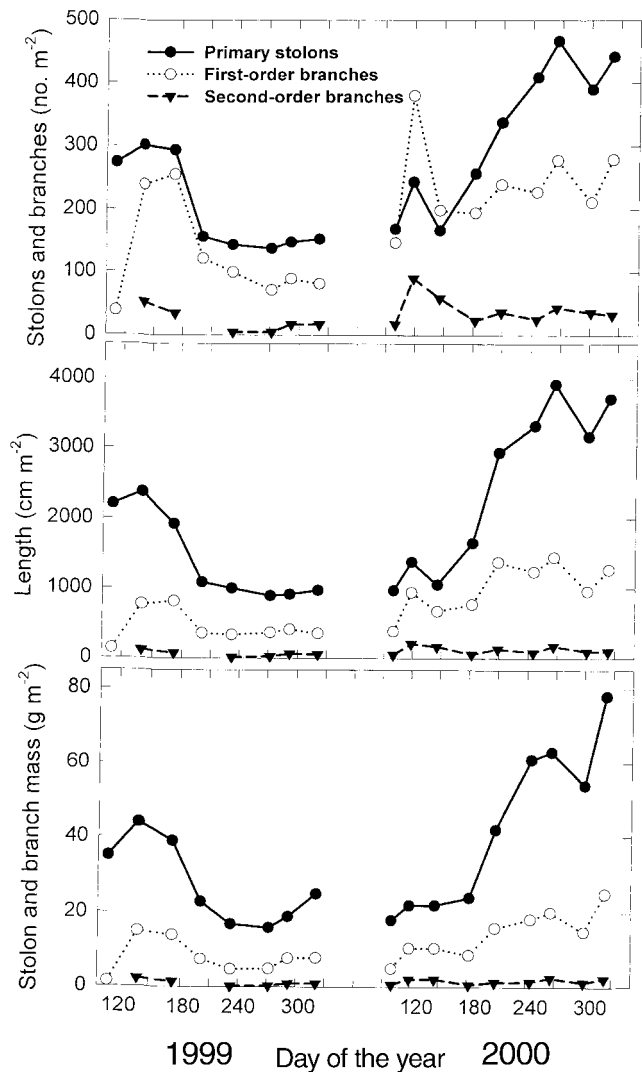

Fig. 2. Changes in the number, length, and mass of primary stolons and first- and second-order stolon branches of white clover during 1999 and 2000. Data are untransformed means. The transformed means along with the SE are in Table A1.
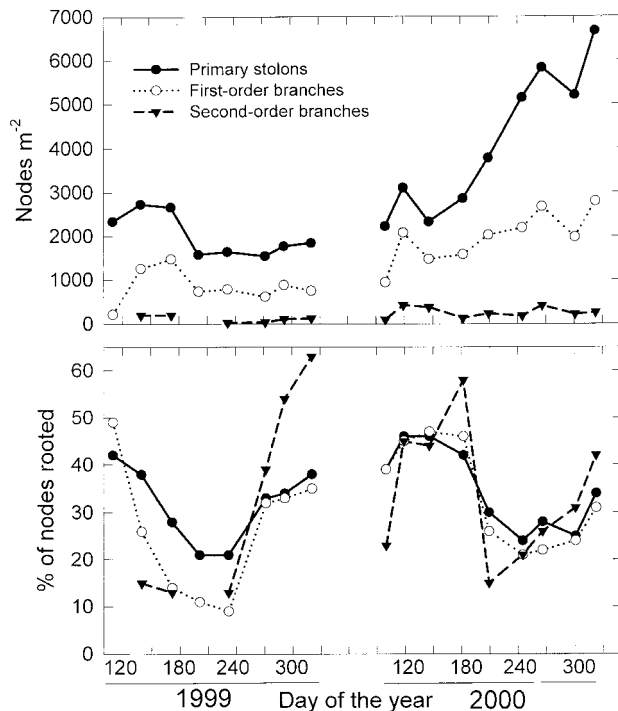
Fig. 3. Density of white clover nodes on primary stolons and branches and the percentage of nodes with roots during 1999 and 2000. Data are untransformed means. The transformed means along with the SE are in Table A2.

tion; however, the length and mass of primary stolons was much greater than that of first- or second-order branches. The number, length, and mass of primary stolons and first-order branches decreased by 40 to 50% during June and July of 1999. However, the total dry mass of stolons increased during September to November, probably because of increased rainfall. The increase in stolon mass with little change in the length or number of stolons may indicate that white clover was accumulating reserve compounds during this time (Collins and Rhodes, 1995).

The pattern of white clover stolon growth and development differed in 2000 compared with 1999. The num-
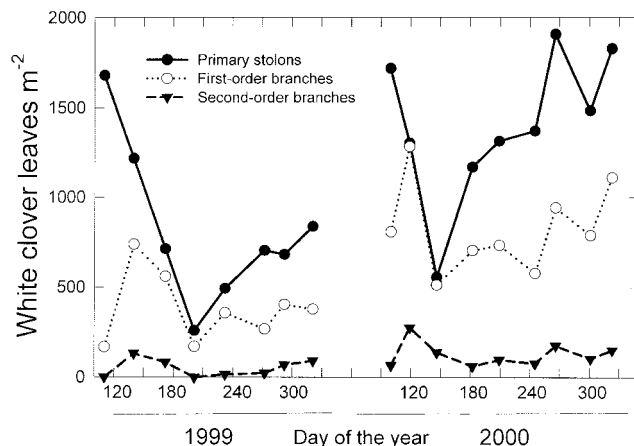

Fig. 4. Density of white clover leaves on primary stolons and first-order branches in 1999 and 2000. Data are untransformed means. The transformed means along with the SE are in Table A3.
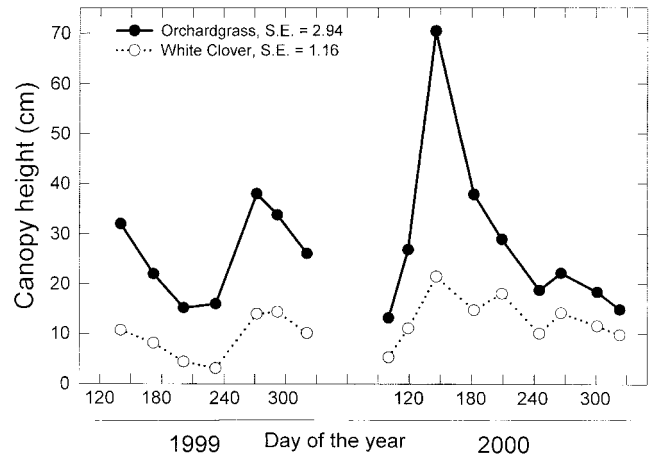

Fig. 5. Canopy height of orchardgrass and white clover during 1999 and 2000. These data did not require transformation.

ber, length, and mass of all stolon classes were nearly the same in early April of 2000 as in late November of 1999, indicating good survival of the stolon populations during the winter (Fig. 2). Primary stolons dominated the white clover population in 2000 as they did in 1999. The number and length of primary stolons doubled from May to September, whereas stolon mass increased threefold. The number of first-order branches peaked in late April of 2000, followed by a second peak in September. Length and mass of first-order branches nearly doubled during the grazing season of 2000.

We observed similar patterns in the number of nodes and rooted nodes (Fig. 3). Although the total number of nodes on primary stolons decreased by ≈50% during May to July 1999, the number of rooted nodes decreased by ≈70% during the same period. The percentage of rooted nodes on primary stolons and first-order branches increased during the fall, reflecting the improved soil moisture conditions (Fig. 1). The percentage of rooted nodes on primary stolons and second-order branches were higher in 2000 than in 1999. The percentage of rooted nodes on primary stolons and first-order branches decreased from May to August and increased somewhat during the fall. There were few second-order branch nodes, thus small changes in the number of rooted nodes resulted in wide swings in the percentage of rooted nodes.

The number of white clover leaves decreased linearly from 1800 m^{-2} in April to 200 live leaves m^{-2} in July 1999 (Fig. 4). The number of leaves m^{-2} increased linearly from July to November with the suspension of grazing and the return of rain in the fall. The number of leaves m^{-2} decreased linearly from early April to May 2000, followed by a large increase in leaves similar to the pattern observed in 1999. The decrease in leaves in 2000, however, probably was because of the very tall canopy of orchardgrass during that time (Fig. 5). The tall orchardgrass canopy may have shaded the clover and reduced leaf production (Frame et al., 1998). In 1999, the orchardgrass canopy decreased in height during the spring and probably did not compete excessively with the white clover for light.
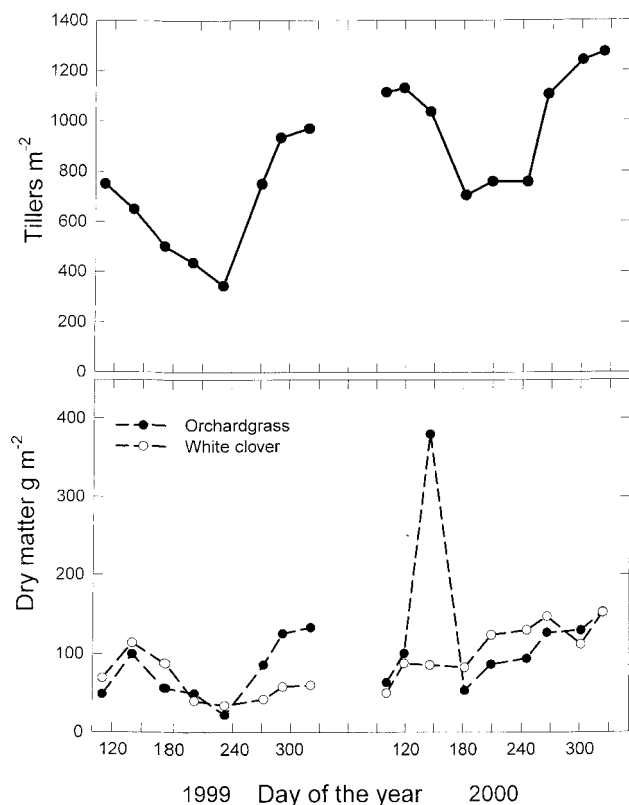
Fig. 6. Density of orchardgrass tillers and dry matter yield of orchardgrass and white clover. White clover yield is the total of leaf and stolon. Data are untransformed means. The transformed means along with the SE are in Table A4.

The density of orchardgrass tillers and the dry matter yield of orchardgrass and white clover followed patterns similar to that of white clover stolon populations (Fig. 6). Orchardgrass yield was very high in May 2000 because delayed grazing allowed reproductive shoots to elongate. Orchardgrass accounted for most of the total grass-legume yield in late 1999, whereas dry matter yield (leaf + stolon) of white clover was greater than orchardgrass during the summer of 2000.

Blue Weevil Larval Damage to Stolons

Stolon injury from blue weevil larval feeding was relatively high at the start of sampling in July 1999 and declined thereafter (Fig. 7). Peak stolon injury in 2000 occurred in May and June. More than 75% of the primary stolons had feeding tunnels caused by the blue weevil in July and August, but this injury decreased in autumn. The percentage of stolons infested by larvae decreased in autumn probably because of new stolon growth and migration of adult weevils to overwintering sites at this time. Many stolons began growing again in autumn but at right angles to damaged areas of the original primary stolon. Also, we noticed there were no second-order branches in July and August, but they appeared later and had <20% injury.

Living larvae of the blue weevil always oriented themselves toward the distal end of stolons. Several tunnels could occur in the same stolon so that damage in some
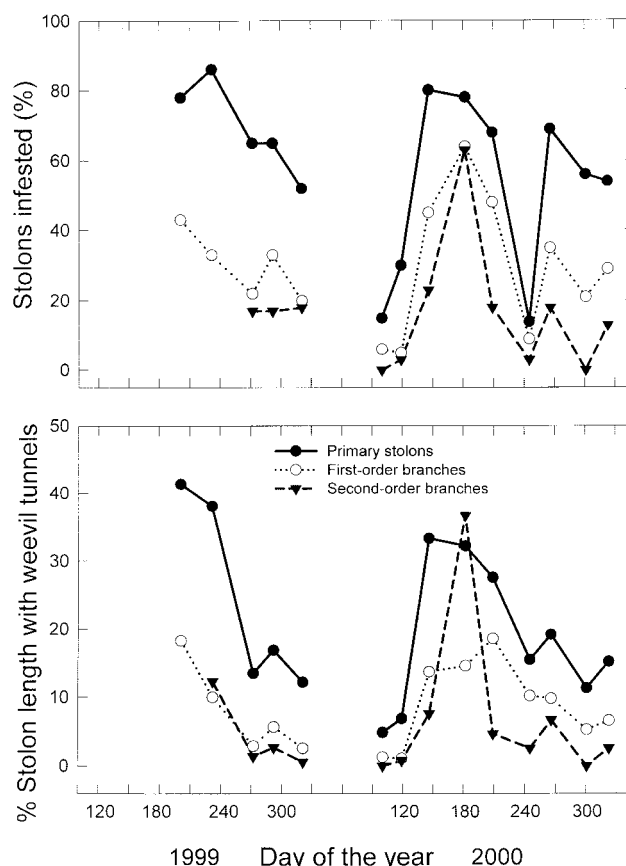

Fig. 7. Blue weevil larval damage to white clover stolons estimated by the percentage of stolons infested and the proportion of stolon length damaged by weevil larvae tunneling inside the stolon. Data are untransformed means. The transformed means along with the SE are in Table A5.

instances occurred along the entire length of the stolon. This indicated that the eggs of the blue weevil could be laid anywhere along the stolon. Tunneling exceeded 35% of the length of infested primary stolons in July and August but declined to ≈15% or less of total length of infested stolons in autumn (Fig. 7). Infested first- and second-order branches had feeding tunnels that were considerably shorter than those in infested primary stolons.

In 2000, 80% of the primary stolons were infested in May and June (Fig. 7). Generally, first- and second-order branches had a lower rate of infestation than primary stolons. The number of stolons infested declined in autumn as in 1999. Two parasitic wasp species were recovered from blue weevil larvae in stolons in 2000. The species were identified by E. Grissell (Systematic Entomology Laboratory, USDA-ARS, Beltsville, MD) as *Homoporus* sp. and *Mesopolobus* sp. (Hymenoptera, Pteromalidae). Both wasps attack several insect species (E. Grissell, 2001, personal communication) and may be a natural control of the blue weevil.

Sitona Larval Damage to Roots

The pattern of *Sitona* damage to roots in 1999 was similar to the pattern of stolon damage by the blue
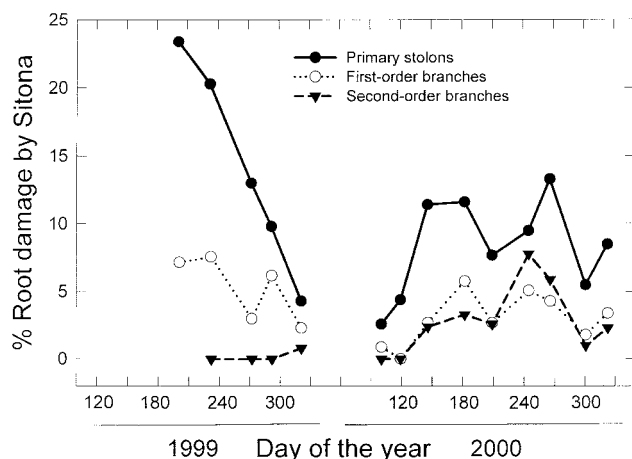
Fig. 8. *Sitona* **larval damage to white clover roots estimated by the percentage of stolons infested and the proportion of root damaged by** *Sitona* **feeding. Data are untransformed means. The transformed means along with the SE are in Table A5.**

weevil (Fig. 7, 8). In 2000, root damage remained at a relatively high level from May to September. More than 20% of root surface was damaged by *Sitona* larvae in July and August on roots originating from primary stolons (Fig. 8). Less injury was found on roots on first-order branches and no injury was noted on roots of second-order branches in 1999. The percentage of root surface injured in 2000 was much lower, ≈13% on roots on primary stolons in June and September (Fig. 8). Roots on first- and second-order branches had less damage than roots on primary stolons during the year. The low amount of *Sitona* injury in 2000 compared with 1999 may have resulted from greater plant growth in 2000 because of the favorable growing conditions.

DISCUSSION AND CONCLUSIONS

Stolon complexity in our study was lowest during the midsummer when temperatures were high and soil moisture limited. This contrasts with research from milder climates in New Zealand and Wales, where white clover plants growing in mixture with perennial ryegrass were smaller and less competitive in the spring (Brock et al., 1988; Fothergill et al., 1997). Our results agree with research from central New York, which indicated that dry, hot weather during the summer limited white clover survival in mixture with orchardgrass (Karsten and Fick, 1999).

Soil water supply can profoundly affect stolon populations. High temperatures and low soil moisture restricted white clover branching and rooting during summer in grazed hill pastures of New Zealand (Chapman, 1983). In that study, the frequency of rooted nodes decreased from 21% of the total number of nodes in the spring to 9% in the summer. Branching frequency decreased from 29 to 21% during the same period. Entire

stolons died more frequently in summer as well. In the southeastern USA, white clover growing in mixture with tall fescue (*Festuca arundinacea* Schreb.) had greater stolon length and mass during June when rainfall was generally higher (Brink and Pederson, 1993). A 30% decrease in relative water content of white clover resulted in an 80% decrease in the number of stolons per plant (Belaygue et al., 1996). In a multisite European study, greater leaf area index of white clover in autumn was attributed to greater rainfall, resulting in greater production of nodal roots in moist soil, which enhanced stolon branch production (Wachendorf et al., 2001b).

Biotic stress from diseases and insects was not reported in the New Zealand and Wales studies (Brock et al., 1988; Fothergill et al., 1997). Fungal pathogens, however, may have damaged stolons in New York (Karsten and Fick, 1999). Our results show that biotic stress from both above- and belowground insect herbivores can seriously damage white clover stolons and roots, compounding abiotic stress from water deficit. The blue weevil larvae damaged stolons by feeding, tunneling, and by chewing exit holes in the stolon. These exit holes provided the sites for stolon decay and fragmentation. Damage to the roots by *Sitona* larvae probably reduced the water uptake ability, N fixation capacity, and aboveground yield of white clover as well (Murray et al., 1996; Murray et al., 2002).

Our data show that the blue weevil can infest most of the stolons of white clover in pastures. The reason for greater numbers of infested primary stolons is not clear, but it may be related to the length of time that stolons are exposed to infestation. Primary stolons are more abundant and are exposed longer to adult weevils for egg laying in the spring than the smaller first- and second-order branches. The interaction of blue weevil feeding stress on stolons with other contributing factors that limit growth such as moisture stress, plant competition, and *Sitona* feeding on roots needs to be considered. The injury from *Sitona* larvae was greater in a dry year. The injury from the blue weevil larvae was similar between years but the white clover compensated for the loss better in 2000 because of the favorable growing conditions.

Despite severe drought stress and chronic insect damage to white clover stolons, the stolon populations recovered dramatically when rainfall returned. Results from our study suggest that climate and biotic stresses are major factors controlling the oscillations of white clover abundance in the northeastern USA. Management to maintain a greater length and mass of stolons will aid the persistence and competitiveness of white clover.

APPENDIX

Tables A1 through A5 contain means of the transformed data along with standard errors and ANOVA results referred to in Fig. 2, 3, 4, 6, 7, and 8.

Table A1. Means of transformed data for length, number, and mass of primary stolons, first- and second-order branches.

Month	Length			Number			Mass		
	Primary stolon	First-order branch	Second-order branch	Primary stolon	First-order branch	Second-order branch	Primary stolon	First-order branch	Second-order branch
		cm m^{-2}			No. m^{-2}			g m^{-2}	
					1999				
April	46.4†	10.6†	−1.001‡	16.4†	5.6†	−1.000‡	6.25†	−0.644‡	−0.997§
May	48.2	25.3	−0.380	17.1	14.1	−0.417	7.05	−0.339	−0.310
June	41.9	25.4	−0.725	16.5	14.4	−0.732	6.39	−0.327	−0.702
July	32.3	18.6	−1.000	12.3	10.8	−1.000	5.09	−0.343	−1.000
August	29.0	15.3	−0.908	11.2	8.6	−0.914	4.14	−0.530	−0.900
September	28.4	16.2	−0.824	11.4	7.3	−0.838	4.09	−0.543	−0.831
October	28.9	18.6	−0.554	11.7	8.7	−0.596	4.45	−0.418	−0.503
November	29.0	13.1	−0.723	11.6	6.6	−0.740	4.82	−0.632	−0.503
					2000				
4 April	30.1	18.4	−0.485	12.4	11.5	−0.525	4.39	−0.467	−0.477
24 April	36.4	29.5	−0.365	15.2	19.0	−0.384	4.99	−0.313	−0.341
May	30.1	24.3	−0.271	12.0	13.3	−0.305	4.65	−0.353	−0.2
June	37.0	23.3	−0.613	15.4	12.0	−0.634	4.65	−0.477	−0.571
July	53.2	33.5	−0.545	18.1	14.4	−0.580	6.86	−0.325	−0.539
August	56.2	33.8	−0.295	19.5	14.4	−0.373	8.20	−0.242	−0.266
September	61.0	35.6	−0.384	21.4	15.7	−0.413	8.30	−0.269	−0.404
October	54.8	29.2	−0.306	19.2	13.7	−0.335	7.70	−0.300	−0.268
November	59.0	34.7	−0.143	20.6	16.3	−0.206	9.22	−0.194	−0.062
SE	3.81	4.01	0.145	1.54	1.82	0.1391	0.643	0.0652	0.135
					F values from ANOVA				
Year	34.8**	33.4*	29.8**	22.7**	31.5**	28.8**	16.4**	21.1**	31.6**
Month (year)	8.4**	2.0	1.6	4.1**	2.4*	1.5	6.0**	3.0*	2.5

* $P < 0.05$.
** $P < 0.01$.
† Data were square-root transformed.
‡ Data were transformed as $-1/\sqrt{y + 1}$.
§ Data were transformed as $-1/(y + 1)^6$.

Table A2. Means of transformed data for the number of nodes m^{-2} and the percentage of nodes with roots.

Date	Number of nodes			% of nodes rooted		
	Primary stolon	First-order branch	Second-order branch	Primary stolon	First-order branch	Second-order branch
		No. m^{-2}			%	
			1999			
April	13.12†	5.31†	−1.00‡	6.44†	6.70†	
May	13.74	9.70	−0.43	6.12	5.09	3.16†
June	13.25	10.27	−0.74	5.24	3.74	3.42
July	11.44	8.87	−1.00	4.52	3.14	
August	10.94	7.64	−0.91	4.48	2.68	3.87
September	11.10	6.87	−0.84	5.68	5.39	5.99
October	11.42	8.79	−0.59	5.79	5.66	7.06
November	11.43	6.01	−0.74	6.02	5.36	7.60
			2000			
4 April	12.55	9.37	−0.53	6.19	6.14	4.11
24 April	14.28	12.38	−0.41	6.73	6.54	6.53
May	12.25	10.70	−0.31	6.78	6.76	6.48
June	13.03	9.42	−0.63	6.29	6.70	7.44
July	15.31	11.56	−0.59	5.41	4.75	3.58
August	16.62	12.34	−0.36	4.90	4.40	3.64
September	17.72	13.06	−0.42	5.30	4.56	4.53
October	16.83	11.88	−0.34	4.96	4.81	5.26
November	18.27	13.89	−0.18	5.83	5.51	6.34
SE	1.013	1.188	0.14	0.268	0.55	1.02
			F values from ANOVA			
Year	47.4**	41.0**	40.8**	9.1**	4.7*	9.7**
Month (year)	2.5*	3.3*	2.0	7.8**	7.3**	4.4**

* $P < 0.05$.
** $P < 0.01$.
† Data were cube-root transformed.
‡ Data were transformed as $-1/\text{cube root } (y + 1)$.

Table A3. Means of transformed data for the number of white clover leaves on stolons.

	1999			2000		
Date	Primary stolon	First-order branch	Second-order branch	Primary stolon	First-order branch	Second-order branch
			No. m^{-2}			
April	40.22†	11.82†	−1.001‡	38.61†	26.86†	−0.547‡
May	34.32	24.85	−0.374	35.20	34.73	−0.347
June	25.97	21.88	−0.725	22.15	21.38	−0.276
July	15.62	12.69	−1.000	35.61	25.21	−0.558
August	20.33	15.24	−0.908	35.69	23.10	−0.311
September	25.53	14.15	−0.822	43.38	28.68	−0.356
October	24.52	18.51	−0.546	37.40	26.43	−0.286
November	27.17	13.58	−0.717	41.67	31.72	−0.115
SE	3.195	3.282	0.1438	3.195	3.282	0.1438
		F values from ANOVA				
Year	34.5**	41.0**	30.2**			
Month (year)	4.7**	1.9	1.8**			

* $P < 0.05$.
** $P < 0.01$.
† Data were square-root transformed.
‡ Data were transformed as $-1\sqrt{(y + 1)}$.

Table A4. Means of transformed data for the number of grass tillers and clover dry matter yields.

	1999			2000		
	Grass		White clover	Grass		White clover
Date	Tillers	Dry matter	Leaf + stolon	Tillers	Dry matter	Leaf + stolon
	No. m^{-2}	g m^{-2}		No. m^{-2}	g m^{-2}	
April	2.91†	1.70†	8.71‡	3.05†	1.81†	7.17‡
May	2.87	1.98	11.22	3.07	2.00	9.83
June	2.74	1.73	9.59	3.04	2.58	9.53
July	2.66	1.68	6.67	2.89	1.95	11.68
August	2.58	1.37	5.61	2.88	1.92	12.05
September	2.89	1.94	6.68	3.07	2.14	12.85
October	2.95	2.09	7.80	3.10	2.12	11.09
November	3.03	2.16	7.50	3.14	2.21	13.08
SE	0.074	0.086	0.977	0.074	0.086	0.977
		F values from ANOVA				
Year	24.8**	28.2**	31.8**			
Month (year)	3.3*	8.7**	3.8**			

* $P < 0.05$.
** $P < 0.01$.
† Data were $\log_{10}$ transformed.
‡ Data were square-root transformed.

Table A5. Means of transformed data for the length of stolon damaged by blue weevil larvae and the percentage of roots damaged by curculio larvae.

	Blue weevil damage			Curculio damage		
Date	Primary stolon	First-order branch	Second-order branch	Primary stolon	First-order branch	Second-order branch
	damaged tunnel length as % of stolon length			%		
		1999				
July	1.617†	1.227†		4.70§	2.34§	
August	1.575	0.846	−0.230‡	4.34	2.52	
September	1.138	0.434	−0.789	3.32	1.18	
October	1.148	0.583	−0.845	3.02	1.96	
November	0.990	0.378	−0.631	1.76	1.08	0.17§
		2000				
4 April	0.492	0.250	−1.005	1.27	0.47	
24 April	0.806	0.278	−0.810	1.90	0.04	0.02
May	1.497	1.052	−0.392	3.06	1.33	1.04
June	1.428	1.338	−0.275	3.06	1.86	1.25
July	1.446	1.148	−0.557	2.72	1.29	1.48
August	1.147	0.952	−0.628	2.92	1.98	1.90
September	1.266	0.859	−0.607	3.35	1.54	1.42
October	1.048	0.630	−1.000	2.20	1.22	0.65
November	1.154	0.817	−0.861	2.80	1.61	0.76
SE	0.1094	0.1306	0.190	0.367	0.37	0.65
		F values from ANOVA				
Year	5.6*	6.1*	2.5	32.4**	16.9**	6.1*
Month (year)	10.1**	8.2**	7.2**	6.8**	5.5**	2.8*

* $P < 0.05$.
** $P < 0.01$.
† Data were cube-root transformed.
‡ Data were transformed as $-1/$cube root $(y + 1)$.
§ Data were square-root transformed.

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